ABSTRACT

Kateřina Muráriková

Influencing of production of plant tissue culture of *Trifolium pratense* L. I

The aim of this thesis was to check the influence of cinnamic acid, as a precursor of the phenylpropanoids biosynthesis, on the flavonoids production of *Trifolium pratense* L. suspension culture, to compare two varieties of this plant, the DO-8 one and the Tempus one, in terms of the effect of this precursor, and to help achieve the successful increase of the production of secondary metabolites by the *Trifolium pratense* L. explant cultures. Concerning the DO-8 variety, the changes of the isoflavonoids production as a result of the cinnamic acid influence have been also tested.

The suspension cultures were being cultivated on the Gamborg's nutrient medium with the addition of 2 $mg.l^{-1}$ 2,4-dichlorphenoxyacetic acid and 2 $mg.l^{-1}$ 6-benzylaminopurine. The cultivation was being realised on a slow moving roller at temperature of 25 °C and in the period of 16 hours of light and 8 hours of dark. The cinnamic acid was added in the four concentrations and its influence was observed after 6, 24, 48 and 168 hours. The flavonoids determination was being applied on the check samples and the samples with the addition of cinnamic acid by the spectrophotometric method in agreement with the Český lékopis 2009 and the isoflavonoids determination by the HPLC method.

The highest content of flavonoids (0.4107 %), which was taken 24 hours after the influence of the 10 $mmol.l^{-1}$ cinnamic acid, was measured in the *Trifolium pratense* L. culture, the variety DO-8. The statistically significant increase of 98 % was realised.

The production of isoflavonoids was most influenced by 100 $mmol.l^{-1}$ cinnamic acid after 48 hours when the content of genistin was increased by 800 % and the daidzein one by 300 %; moreover, the content of genistein and biochanin A was, in comparison with the control, found out.